

Dynamics of Agricultural Biotechnology

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and Ni²⁺ were poor substitutes. This enzyme also required these divalent cations to stabilize its structure and function under extreme conditions such as high and low temperatures and urea denaturation. The glutamate analog L-methionine-D,L-sulfoximine, inactivated the enzyme, whereas the GOGAT inhibitor, azaserine, had no effect on the enzyme activity.

123 GOYAL, DINESH. 1992. **A simplified method for screening and characterization of plasmid DNA in cyanobacteria.** *Journal of Microbiological Methods*, 15: 1, 7-15; 29 ref.

A simplified method of studying plasmid distribution in cyanobacteria involves direct agarose gel electrophoresis of heat-treated, ethanol-precipitated, plasmid preparations from the cleared lysates without ultracentrifugation. The method is sensitive and can be used to determine the number of different plasmid species and their molecular weights from the agarose gel patterns. The results compare well with those obtained by the CsCl-EtBr equilibrium density centrifugation technique.

124 TREHAN, K; SINHA, U. 1982. **DNA-mediated transformation in *Nostoc muscorum*, a nitrogen-fixing cyanobacterium.** *Australian Journal of Biological Sciences*, 35: 5, 573-577; 11 ref., 4 tab.

Genetic transformation of an auxotrophic valine-requiring marker and a marker with resistance to p-fluorophenylalanine has been demonstrated in *Nostoc muscorum*. Transformation is primarily mediated by DNA and is insensitive to ribonuclease and proteinase. The kinetics of the frequency of transformation, which is dependent on the concentration of DNA, suggests a saturation phase. Transformants, though devoid of heterocysts, are able to grow in a medium lacking a combined nitrogen source.

125 VENKATARAMAN, GS. 1985. **Molecular biology and biotechnology of cyanobacterial nitrogen fixation.** *Current Science*, 54: 11, 493-498; 68 ref.

ALGAE, *SPIRULINA PLATENSIS*

126 FATMA, T. 1990. **Effect of culture filtrate on growth of *Spirulina platensis*.** *Current Science*, 59: 16, 797-798; 3 ref.

Seven levels of *Spirulina* culture filtrate were added to cell cultures of *S. platensis*. From the plots of absorbance against time it is deduced that the culture filtrate contains extracellular growth-stimulatory factors.

BACTERIOLOGY

127 BATISH, VK; GROVER, S; NEELAKANTAN, S. 1992. **Genetic improvement of lactobacilli and their application in food processing.** *Microbiologie, Aliments, Nutrition*, 10: 1, 1-9; 121 ref.

A review of recent developments in the genetic improvement of lactobacilli is presented under the headings: Plasmid biology of lactobacilli; Gene transfer in lactobacilli; Development of cloning vectors; Molecular cloning of *Lactobacillus* genes; and Future prospects for strain improvement. Current and future applications of lactobacilli in dairy & food industries are also discussed.

128 DAVID, BP; PURUSHOTHAMAN, V; VENKATESAN, RA. 1993. **Comparison of molecular weight estimation techniques: bacterial plasmid DNA.** *Indian Journal of Animal Sciences*, 63: 11, 1146-1151.

129 DHARMSTHITI, S; KRISHNAPILLAI, V. 1993. **DNA sequence conservation at the gene level in a conserved chromosomal segment in two *Pseudomonas* species.** *Journal of Genetics*, 72: 1, 1-14.

130 GANDHI, DN; NAMBU DRIPAD, VKN. 1981. **Antagonistic effect of cell free culture filtrate and isolation of antibiotic from *Lactobacillus acidophilus*.** *Indian Journal of Dairy Science*, 34: 1, 98-101; 11 ref.

Sterilized skim milk was inoculated with *Lactobacillus acidophilus* R and incubated at 39°C for 24 h. The culture was then centrifuged and the supernatant Seitz filtered. Antibacterial activity was extracted from the filtrate with methanol and acetone followed by Sephadex G 25 gel filtration. The extract inhibited growth of *Escherichia coli*, *Micrococcus flavus*, *Staphylococcus aureus* and *Salmonella weltevreden*. The antibacterial activity was stable at low pH and resistant to heating at 100°C for 20 min; it can be stored at -25°C for 6 months without loss of activity.

131 GARG, SK; MITAL, BK. 1992. **Genetics of antagonistic action and drug resistance in *Lactobacillus acidophilus*.** *World Journal of Microbiology and Biotechnology*, 8: 2, 92-97; 70 ref.

Lactobacillus acidophilus has been recommended as a dietary adjunct because of its antagonistic action toward intestinal pathogens, and anti-carcinogenic and hypocholesterolaemic activities. Many *L. acidophilus* strains harbour plasmids and such strains generally produce bacteriocin(s). Resistance to antibiotics has also been shown to be linked with plasmids. Gene transfer and

cloning systems are being developed for *L. acidophilus*, which should permit the rapid genetic characterization of desired species and their modification to obtain predetermined traits. Drug resistance determinants and production of antibiotic-like substances may serve as suitable markers for the study and development of these genetic systems. Recent developments in gene transfer systems have been reviewed here.

132 PRASAD, JRK; SINHA, PR; SINHA, RN. 1991. Protoplast fusion in selected *Lactobacillus* species. *Microbiologie, Aliments, Nutrition*, 9: 1, 69-75; 14 ref.

Protoplast fusion was carried out using *Lactobacillus acidophilus* R. *L. casei* 300 and *Lactobacillus sp.* (L4) using antibiotic resistance as markers. Antibiotic sensitivities against 10 antibiotics were determined using antibiotic discs under anaerobic conditions. Though there was variation in the sensitivity of 3 cultures to a particular antibiotic, they were all resistant to streptomycin and sensitive to ampicillin and rifampicin. To isolate fusants of *L. acidophilus* R and *Lactobacillus sp.* (L4) as well as *L. acidophilus* R and *L. casei* 300 Tetr Eryr markers were used. In the case of fusion between *Lactobacillus sp.* (L4) and *L. casei* 300, Novr Kan r markers were used. The regeneration frequencies on the basis of total cell input ranged between 8×10^{-10} and 10×10^{-8} . Plasmid profiles of parents and fusants indicated the possibility of transfer of genetic material. One of the parents *L. acidophilus* R was plasmid-free and the fusants R-Lc (*L. acidophilus* R-*L. casei*) and R-L4 (*L. acidophilus* R-*Lactobacillus* L4) showed plasmid DNA. This study indicates the possibility of transfer of genetic determinants of lactobacilli using this technique.

133 SHARMA, V; BATISH, VK; GROVER, S. 1992. Evidence of plasmid-linked phenotypic characters in two wild *Lactobacillus casei* strains. *Microbiology, Aliments, Nutrition*, 10: 2, 167-175; 21 ref.

The 2 *Lactobacillus* isolates possessing antibacterial activity identified as *L. casei* Vc and V46 harboured 3 plasmids each approx. 3.2, 4.1, 32.9 and 4.2, 7.8, 35.5 MDa, resp. Among the different curing agents, ethidium bromide appeared to be the best for curing of Lac+ and Prt+ phenotypes in both the cultures. The curing efficiencies of this agent for lactose utilizing (Lac+) phenotype in the 2 cultures were 37.5 and 14.28% and 5 µg/ml at elevated temp. However, ethidium bromide was highly effective at 15 µg/ml for curing proteolytic (Prt+) phenotypes as the corresponding curing efficiencies were 53.91 and 84.74% at 42°C. Although the curing of Lac+ phenotype could also be achieved with acriflavine both at 37 and 42°C at 10 µg/ml concn., it

was not very effective against Prt+ phenotype. Novobiocin was not suitable for curing of Lac+ and Prt+ phenotypes. The curing of sucrose utilizing (Suc+) phenotype was achieved both with acriflavine and novobiocin. A majority of the Lac- and Suc- variants reverted. However, the Prt- variants appeared to be quite stable. The 32.9 and 35.5 MDa plasmids were missing from Lac- variants of Vc and V46 resp. A few Prt- variants of V46 lacked 4.2 and 7.8 MDa plasmids. Most of the Lac- and Prt- variants retained antibacterial activity. In some cases, enhanced antibacterial activity was recorded. However, Suc- variants did not exhibit any antibacterial activity.

134 SRIVASTAVA, A; ROYCHOUHDURY, PK; SAHAI, V. 1992. Extractive lactic acid fermentation using ion-exchange resin. *Biotechnology and Bioengineering*, 39: 6, 607-613; 39 ref.

Since lactic acid production by fermentation is limited by product inhibition, tests were done on adsorptive extraction of the lactic acid generated onto ion-exchange resin. Previously adapted *Lactobacillus delbrueckii* NRRL-B445 was grown anaerobically at pH 6.0 (maintained with 2.5M NaOH) in a 'Bioengineering AG' bioreactor (working capacity 1.6 litres), by batch and extractive techniques, on a medium containing (g/litre): sucrose, 100; yeast extract, 30; sodium sulphate and succinate, 2 each; KH₂PO₄, 0.2; K₂HPO₄, 0.2; MgSO₄.7H₂O, 0.3; MnSO₄.H₂O, 0.03; FeSO₄.7H₂O, 0.03. In extractive mode, when the lactic acid concentration reached 18-19 g/litre, broth was circulated at 5 ml/min through a vertical column (ht 21 cm, dia 4 cm, capacity 120 g) of Amberlite IRA-400 resin in the OH-form; the recycle pump was stopped whenever pH reached 6.1 and restarted at pH 5.9. Experiments at 37-45°C showed clear maxima at 39° for both cell productivity and lactic acid productivity, despite a maximum rate of substrate consumption at 38°. The maximum hourly productivity of lactic acid was 1.665 g/l, compared with 0.313 g/litre in batch mode; sucrose conversion was 100% in 60 h, compared with 85.7% in 196 h.

135 SRIVASTAVA, RANJANA; BHARTI, RAJNISH; SRIVASTAVA, AK. 1990. Characterization of a novel cellobiase from *Bacillus subtilis* and expression of its structural gene in *Escherichia coli*. *Biotechnology Letters*, 12: 7, 541-545; 16 ref.

A strain of *B. subtilis* produced a cellobiase resistant to catabolic repression by glucose. When the structural gene encoding cellobiase was cloned and expressed in *E. coli*, the enzyme produced was resistant to repression by glucose.

136 TABASSUM, R; RAJOKA, MI; MALIK, KA. 1992. Use of chemostat for enhanced production of beta-glucosidase by newly isolated anaerobic cellulolytic *Clostridium* strain RT9. *Applied Biochemistry and Biotechnology*, 34/35, 317-329.

137 TULI, R; SALUJA, J; NOTANI, NK. 1989. Cloning and expression in *Escherichia coli* of entomotoxic protein gene from *Bacillus thuringiensis subsp. kurstaki*. *Journal of Genetics*, 68: 3, 147-160; 39 ref.

In a laboratory experiment, a plasmid-borne larvicidal crystal protein gene from *Bacillus thuringiensis subsp. kurstaki* was cloned in *Escherichia coli* using a specific 20-mer oligonucleotide probe. The gene expressed in *E. coli* at a high level. Transgenic *E. coli* cells produced large irregular bodies which were bright under phase contrast microscopy. These were released by sonic disruption of the cells and pelleted by centrifugation. In toxicity trials against the noctuid *Spodoptera litura*, 3rd-instar larvae were exposed to 35 mm castor (*Ricinus communis*) leaf discs treated with transgenic *E. coli*. An immediate effect was the cessation of feeding. A similar response, but to a lesser extent, was observed in larvae feeding on leaf discs treated with a spore-crystal supernatant preparation of *B. T. subsp. kurstaki*. Larvae showed severe growth retardation and no mortality. Larval weight decreased by 80% after feeding for 3 days on leaf discs treated with transgenic *E. coli*. This was greater than the 39% reduction caused by comparable amounts of protein in the spore-crystal preparation. Larval growth was retarded marginally (11%) by the *E. coli* control. The pupation of larvae in controls was complete by the 23rd day; however, the majority of larvae fed on toxin preparations remained in the larval stage. The delay in pupation was in proportion to the extent of the toxicity. Treated larvae required 9 extra days before pupation was complete. Mortality was observed only when the toxin was fed to newly exposed larvae.

FIELD CROPS

138 BAJAJ, YPS. 1983. Haploid protoplasts. *International Review of Cytology*, Suppl. 16, 113-141; 73 ref.

The isolation, culture and fusion of protoplasts from pollen tetrads and maturing pollen, the isolation of haploid protoplasts from mesophyll and callus cells, and the subsequent regeneration of these protoplasts into entire plants is described and a summary of this work is given in a table, comprising many plant species and including *Brassica napus*, *Nicotiana spp.*, potato, rice, wheat and oats. The implications and prospects of

haploid protoplasts in the induction of genetic variability, mutations and somatic cell genetics are considered.

139 JALALI, SK; SINGH, SP; BALLAL, CR. 1987. Role of the host plants of *Spodoptera litura* (Fabricius) on the degree of parasitism by *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *Indian Journal of Agricultural Sciences*, 57: 9, 676-678; 3 ref.

Laboratory studies were conducted in India to determine the effect of host plants on the degree of parasitism of the noctuid *Spodoptera litura* by *Cotesia marginiventris* [*Apanteles marginiventris*]. Results of single-plant choice tests indicated that *A. marginiventris* had a marked preference for *S. litura* larvae on castor (*Ricinus communis*) (49.3% parasitism), followed by cowpea (*Vigna unguiculata*) (24.0%), tobacco (20.0%), okra (16.0%), cabbage (13.3%), knolkhol [kohlrabi] (10.7%), cauliflower (10.7%) and beetroot (9.3%). Although 20% of the larvae were parasitized on tobacco leaves, female parasitoids became very inactive after contact with the leaves and died within an hour. The sex ratio of *A. marginiventris* differed slightly on the various host plants. Results of multiple-plant choice tests indicated that given a choice of all the host plants at once, the parasitoid preferred the host on kohlrabi (56% parasitism), followed by cabbage (29.3%), castor (28.0%), cowpea (28.0%), beetroot (18.7%), cauliflower (16.0%), okra (10.7%) and tobacco (1.3%). In this test, the parasitoid showed least preference for larvae on tobacco. These results indicate that *A. marginiventris* would not be suitable for release against *S. litura* on tobacco.

140 MIAH, MAA. 1992. Tissue culture; advances in crop science. *Proc. on the First biennial Conference of the Crop Science Society of Bangladesh*. (Dhaka: May 18-20). Crop Science Society of Bangladesh. p. 294-299.

CEREAL GRAINS

141 AZIZ, JA; AZIZ, SA. 1985. Food preference and the plant selection pattern in *Oxya velox* Fab. (Orthoptera: Acrididae). *Journal of Entomological Research*, 9: 2, 179-182; 7 ref.

The food preference of *Oxya velox* was evaluated in the laboratory at 30°C and 60% RH using 10 different plant leaves. The early instar hoppers preferred the grass to the cereal crops, while the reverse was found for late instar hoppers and adults. The descending order of preference for late instar hoppers and adults was a mixed diet of rice, *Cynodon dactylon* and *Echinochloa*